REMARKS/ARGUMEMNTS

By virtue of this Amendment, Claims 5 and 45 have been cancelled. Claims 7, 18, 41, 60, 61, 62, and 76 have been amended to particularly point out and distinctly claim the subject matter. Support for these amendments can be found in the original claim language, and/or in the specification as original filed. New claim 77 has been added. The recitation of "all" outer-surface proteins necessary for packaging the phage particle in claim 77 is supported by paragraphs 120 and 121 of the specification as originally filed.

No new matter is introduced. Entry of this Amendment is respectfully requested. Upon entry of this paper, claims 1, 6-7, 10-20, 41, 42, 47-54, 56, 57, 59-62 and 64-77 are now pending.

I. Claim Objection

Claim 5 is objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Claim 5 has been cancelled. Therefore, this objection is moot.

Claims 7 is objected to because of an editorial error in the phrase "wherein in the outer-surface sequences." The error has been corrected by deleting the word "in".

Claim 45 is objected to under 37 CFR 1.75(c), as being improper dependent form for failing to further limit the subject matter of a previous claim. This claim has been cancelled. Therefore, the objection is obviated.

Claim 61 is objected to because of an editorial error. The word "the" is inserted prior to the phrase "method of claim 60" as suggested by the Examiner. Thus, this objection has been overcome.

II. Claim Rejections Under 35 U.S.C. §112, Second Paragraph:

Claims 41-42, 45, 47-53, 56, 59, 61 and 76 are rejected under 35 U.S.C. §112, second paragraph, as, allegedly, being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

Specifically, claim 41 and its dependents are rejected for reciting the phrase "producing a polypeptide within or on the outer surface of a phage particle." Applicants have amended the phrase to recite "producing a polypeptide on the outer surface of a phage particle." This amendment eliminates the alleged ambiguities, if any, in claim 41 and the claims dependent therefrom including claims 42, 47-53 and 59. As noted above, claim 45 has been cancelled.

Claim 61 is rejected as, allegedly, being vague for not specifying whether the displayed polypeptide is attached to a phage particle. Claim 61 has been amended to specifically recite this aspect according to the Examiner's suggestion. This rejection is therefore moot.

Claim 76 is rejected for, allegedly, as being unclear as to whether the term "functional motif thereof" necessarily applies to all members of the Markush group. Claim 76 has now been amended to require that the functional motif apply to "any one of the members of the group." Therefore, this rejection has been obviated.

III. Claim Rejections Under 35 U.S.C. §102:

The Action cites several references as, allegedly, anticipating certain claimed subject matter. Applicants respectfully traverse each rejection for the following reasons.

a. Krebber et al. (FEBS Letters, 1995, Vol.1 377, pages 227-231)

Claims 62 and 64 that are drawn to phage particles having the specified structural features stand rejected under 35 U.S.C. §102(b) in view of Krebber et al. The Examiner is of the opinion that the displayed protein in Krebber et al. is the amino-terminal portion of the gIII protein. In particular, the first paragraph of the Action at page 8 states the following: "In the examples taught by Krebber et al., the amino-terminal portion of the gIII protein is displayed via this non-covalent interaction to

yield an infectious particle." Without conceding to this interpretation, Applicants have amended claim 62 and hence its dependent claim 64 to specify that the displayed polypeptide is exogenous, i.e., non-phage, as indicated by the Examiner. This amendment distinguishes Krebber's particle that displays, according to the Examiner, a portion of gIII, which is a phage protein.

b. Duenas et al. (FEMS Microbiology Letters, 1995, Vol. 125, pages 317-322) and Borrebaeck (U.S. Patent No. 6,027,930)

Claims 41-42, 45 (now canceled), 48, 56, 59, 62 and 64 stand rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Duenas et al. (FEMS Microbiology Letters, 1995, Vol. 125, pages 317-322) or Borrebaeck (U.S. Patent No. 6,027,930). The Examiner opined that this rejection would be overcome by specifying that the displayed polypeptide is "exogenous" (i.e., a non-phage protein)[1]. Accordingly, the independent claims 41 and 62, and hence their respective dependents (claims 42, 48, 56, 59, and 64) have been amended pursuant to the Examiner's suggestion. Withdrawal of this rejection is respectfully requested.

c. Lohning et al. (WO 01/05950 A2)

Claims 1, 5-7, 10-11, 18, 41-42, 45, 47, 54, 56-57, 59-62 and 64-76 are rejected under 35 U.S.C. §102(a) as, allegedly, being anticipated by Lohning et al. (WO 01/05950 A2). Applicants respectfully disagree.

Lohning et al. discloses two types of phage display systems, namely the "one-vector system" and the "two-vector system," for expressing a singe chain scFv fragment. Both systems are structurally distinct than the claimed adapter-directed display system. The one-vector system contains a single dicistronic plasmid, in which the phage coat gene gIII inserted with an extra cysteine codon is controlled by one promoter, and the scFv-hag2 fragment inserted with another

1 First paragraph of the Action at page 9 states the following: "However, with regard to this particular rejection, it would be remedial to amend the claims to include the limitation, as is explicitly recited in claim 1, that the protein that is displayed due to the interaction between the first and second adapters is an "exogenous protein" (i.e. a non-phage protein) that is displayed according to the method of claim 60."

cysteine codon is controlled by a different promoter (*see* the section at page 27, entitled "Construction of vectors expressing scFvs and engineered phage coat proteins"). To display the scFv-hag2 fragment, a helper vector VCSM13 carrying all of the widetype phage coat proteins is used to rescue the dicistronic phagemid (*see*, Figure 7a and the section at page 28, entitled "Attachment of scFvs to phage coat proteins via disulfide bond").

This one-vector system differs from the adapter-directed display system of claim 1 in at least two regards. First, the dicistronic phagemid contains gIII, a gene encoding a functional outer-surface phage protein that directs the packaging or assembly of the exogenous scFv-hag2 displayed on the phage surface (*see*, Figure 7a). By contrast, the claimed adapter-directed display system employs an expression vector "devoid of outer-surface sequences encoding functional outer-surface proteins of the phage particle." This element is missing in Lohning's one vector system.

Second, the one-vector system does not have a helper vector as instantly claimed. As mentioned above, Lohning employed the helper vector VCSM13. *See*, Lohning et al. at page 37 stating that "Phages were produced using helper phage VCSM13 following standard protocols (Kay et al. 1996)." According to Kay et al. 1996, VCSM13 is a M13 helper phage that contains all of the widetype phage outer-surface proteins for packaging and displaying exogenous polypeptides. For example, page 35 of Kay et al. describes that M13 helper phage carries "the full complement of capsid-encoding genes...", and page 39 depicts all outer-surface proteins I through VIII in the helper vector. Both pages of Kay et al. are attached herewith for the Examiner's convenience. Therefore, Lohning et al. does not teach or even suggest engineering the helper vector, and it certainly does not describe engineering it such that "at least one of the outer-surface proteins is fused in-frame to a second adapter." As such, this element is missing in Lohning's one-vector system. It is also lacking in Lohning's two-vector system discussed below.

The two-vector system contains one vector carrying the gIII sequence inserted with an extra codon for a cysteine residue, and another vector carrying the scFv-hag2 fragment inserted with the pairing cysteine residue (*see*, Figure 6a and the section at page 27, entitled "Construction of vectors

expressing scFvs and engineered phage coat proteins"). To effect the display of the scFv-hag2 fragment, both the scFv-hag2 vector and gIII vector are co-transformed into *E.Coli*, and phage particles are produced using, again, the helper vector VCSM13 (*see*, the section at page 28, entitled "Attachment of scFvs to phage coat proteins via disulfide bond"). This helper vector does not contain a second adapter as instantly claimed, i.e., a second adapter that mediates pairwise interaction with the first adapter (the cysteine residue) contained in the expression vector, namely the scFv-hag2 vector. Rather, Lohning's two-vector system displays the exogenous scFv-hag2 fragment via the formation of a covalent bond between the two cysteine residues: one from the expression scFv-hag2 vector, and the other from the vector coding for the engineered coat protein gIII. However, the claimed adapter-directed system requires that the first adapter reside in the expression vector, and the second adapter reside in the helper vector. It is the pairwise interaction between these two adapters located in these two specified vectors (which are distinct from Lohning's vectors) that cause the display of the exogenous polypeptide.

Moreover, the gIII vector expressing a single outer-surface protein, namely gIII, cannot be considered as the helper vector of the invention display system, because it does not contain outer-surface sequences encoding outer-surface proteins necessary for packaging the phage particle, wherein at least one of the outer-surface proteins is fused in-frame to a second adapter. Indeed, there is no teaching or even a hint of this particular design of a display system in Lohning. Therefore, Applicants respectfully submit that Lohning et al. does not anticipate the adapter-directed system recited in claims 1, 5-7, 10-11, 18, 54, 57, 60-61 and 65-76. Nor does it render the claimed system obvious.

Lohning et al. also does not read on the expression vector of claims 41-42, 47, 56, 59, and the phage particle recited in claims 62 and 64. The independent expression vector claim 41 recites that "expression of the polypeptide on the outer surface of the phage particle is mediated via non-covalent pairwise interaction between the first adapter and a second adapter." Similarly, the independent claim 62 recites that "said first adapter acting, when the fusion polypeptide is produced

in a suitable host cell, to cause the display of the fusion polypeptide via non-covalent pairwise interaction between the first adapter and a second adapter..." On the contrary, Lohning et al. teaches pairing two cysteine residues to form a covalent disulfide bond to effect the display of scFv-hag2. As is apparent to one skilled in the art, the instantly claimed expression vector in claim 41 and its dependents (claims 42, 45, 47, 56, and 59) are different from Lohning's expression vector. For the same reasons, the resulting phage particles described in claims 62 and 64 are also distinct from Lohning's phage particles.

Taken together, Lohning discloses an entirely different display system. The individual components of this system are structurally distinct and mechanistically different from the claimed invention. Applicants respectfully request withdrawal of this rejection.

d. Zucconi et al. (Journal of Molecular Biology, 2001, Vol. 307, pages 1329-1339)

Claims 1, 5-7, 12, 18, 41, 45, 48, 51, 54, 56-57, 59-62, 64-67, 69 and 71-76 stand rejected under 35 U.S.C. 102§(a) as, allegedly, being anticipated by Zucconi et al. The Action at page 11 states the following:

> "Zucconi et al. teach methods of selecting ligand by panning cDNA domain libraries displayed as fusion proteins with capsid protein D on phage a (e.g., Abstract). In one set of panning experiments a fusion protein (GST-Syn) comprising a fragment of the praline-rich domain (PRD) of synaptojanin 1 and glutathione S-transferase (GST) was expressed and immobilized to a glutathione-Sepharose solid support. This affinity resin was panned with cDNA libraries from human brain whose products were displayed on the surface of phage λ as fusion protens with gpD (e.g. page 1331, column 2)....In these experiments, the GST-Syn fusion protein can be considered as the fusion protein encoded by the expression vector of part (a) of claim 1 in that the PRD domain serves as an adapter that allows the 'display' of the GST fusion. The GST protein in this case has the desirable property of binding glutathione. Zucconi et al. teach further experiments where different GST fusion proteins comprising peptide motifs from within the synaptojanin 1 PRD domain were used as bait for filamentous phage displaying random non-[a]peptides fused to the major coat protein pVIII (e.g., page 1333). The expression vectors used to express the various GST fusion proteins taught by Zucconi et al. were not disclosed as comprising any phage outer-surface sequences encoding

functional outer-surface proteins of the λ particle, nor would there be any reasons to expect such sequences to be present."

Applicants respectfully submit that Zucconi et al. does not teach the adapter-display system of claims 1, 5-7, 10-11, 18, 54, 57, 60-61 and 65-76. It also does not read on the expression vector of claims 41-42, 45, 47, 56, 59. Nor does it even suggest the phage particle recited in claims 62 and 64.

At the outset, Zucconi et al. does not concern the design of a genetic package for displaying exogenous sequences. Rather, as explicitly stated by Zucconi and acknowledged by the Examiner, Zucconi et al. discloses using immobilized GST-Syn fusion protein produced from *E.Coli* as the bait to "pan" existing phage cDNA domain libraries. For example, column 1 at page 1337 states that "The synaptojanine 1 fragment corresponding to residues 1058-119 was expressed in bacteria as a GST-fusion protein, immobilized on glutathione-Sepharose 4B beads (Amersham Pharmacia Biotech), and used as a bait for affinity selection of interacting clones from a human brain cDNA library whose products are displayed on the surface of lambda capsid."

Nothing in this Zucconi system matches to the expression vector or the helper vector embodied in the claimed invention. In particular, Applicants respectfully disagree with the Examiner's characterization that "In these experiments, the GST-Syn fusion protein can be considered as the fusion protein encoded by the expression vector of part (a) of claim 1 in that the PRD domain serves as an adapter that allows the 'display' of the GST fusion."

First, the GST-Syn fusion protein is not designed to be, and certainly is not displayed on the outer surface of a phage particle as required by all independent claims 1, 41, and 62. For example, claim 1 recites "An adapter-directed display system for displaying an exogenous polypeptide on the outer surface of phage particle." The GST-Syn fusion is produced inside the *E.Coli* and is harvested as soluble protein using glutathione beads that bind to the GST portion of the fusion protein (*see*, e.g. column 1 at page 1337). This is a routine protein purification method and not a display system as

understood by artisans in the field[2] and as delineated in the present patent application. Put simply, the GST-Syn fusion is immobilized on beads, and not packaged and displayed on the outer surface of a genetic package, such as a phage particle. The genetic material encoding the GST-Syn fusion also does not reside within the phage particle. The aspect of phage display is recited in all independent claims including claims 1, 41, 62, and hence in all of their respective dependents.

Second, the PRD domain cannot be considered as the first adapter of the claimed expression vector as suggested by the Examiner, because it does not meet the following claim element: "said first and second adaptor acting, when the polypeptide is produced in a suitable host cell, to cause the display of the polypeptide via pairwise interaction between the first and second adapters." In Zucconi et al., the PRD-containing fusion is expressed separately from the cDNA domain libraries with which it is allowed to interact. Specifically, the PRD-containing fusion is expressed and harvested from *E. Coli* as a soluble protein, and the phage cDNA domain libraries are separately displayed on λ particles. As stated in Zucconi et al., the PRD domain is used as a bait to identify PRD ligands via panning that takes place outside a cell, i.e., on the GST beads. Therefore, the PRD domain is not used to cause the display of any polypeptide via pairwise interaction taking place inside a suitable host cell. In fact, there is no second adaptor in the Zucconi system.

Page 12 of the Action also noted that "the expression vectors used to express the various GST fusion proteins taught by Zucconi et al. were not disclosed as comprising any phage outer-surface sequences encoding functional outer-surface proteins of the λ particle, nor would there be any reasons to expect such sequences to be present." However, as explained above, there is no reason to suspect that Zucconi's GST-expression vectors are even applicable for display by phage particles. The GST-expression vectors yield soluble fusion proteins in *E. Coli* for purification with the use of the GST beads.

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2 The New England Biolabs product description describes phage display as the following: "What is phage display? Phage display describes an *in vitro* selection technique in which a peptide or protein is genetically fused to a coat protein of a bacteriophage, resulting in display of the fused protein on the exterior of the phage virion, while the DNA encoding the fusion resides within the virion."

Zucconi's cDNA domain libraries also do not qualify as the expression vectors as instantly claimed. The cDNA domain libraries are expressed as fusions with capsid protein D on phage λ (e.g., Abstract), or as fusions with pVIII, another outer-surface protein of filamentous phage (e.g. page 1333, column 1). Both capsid protein D and pVIII are functional outer-surface proteins that facilitate or direct the packaging or assembly of the exogenous cDNA domains for display on the phage particles. By contrast, the claimed expression vectors are devoid of any sequences encoding functional outer-surface proteins of phage particles. As such, the cDNA domain libraries are not the claimed expression vectors. Similarly, the resulting phage particles do not meet this claimed aspect recited in claims 62 and 64.

Furthermore, the Action does not identified any component in Zucconi's panning system that matches to the helper vector of claim 1. Therefore, the Office has not met its burden to establish that Zucconi teaches each and every element of the claimed subject matter. In particular, claim 1 and its dependents require "a helper vector comprising outer-surface sequences encoding outer-surface proteins necessary for packaging the phage particle, wherein at least one of the outer-surface proteins is fused in-frame to a second adapter." Nowhere in Zucconi teaches or even hints at the construction of such a helper vector for displaying exogenous polypeptides on a phage particle. Indeed, the use of coat-protein fusion constructs suggests against the need for such helper vector, and hence teaches away from the claimed invention.

Finally, the Examiner stated that claim 60 does not explicitly recite that the components of the adapter-directed display system of claim 1 are introduced into the same cell. Applicants have amended claim 60 to recite "a single host cell" to clarify the alleged ambiguity, if present at all. Accordingly, this rejection should be withdrawal.

In sum, Applicants respectfully submit that Zucconi neither anticipates nor renders the claimed subject matter obvious. Withdrawal of this rejection is requested.

CONCLUSION

Applicants believe that the foregoing fully addresses the issues raised in the Action. In light of the foregoing amendments and remarks, it is believed that this application is in condition of allowance. An early Notice of Allowance is earnestly requested.

If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the instant application, the Examiner is encouraged to telephone the undersigned at (650) 849-3383.

It is believed that a fee under 37 C.F.R. §§ 1.17(a)(2) in the amount of $225.00 to cover for a two-month extension of time by a small entity is required. Accordingly, the Commissioner is authorized to deduct said fee and any other fees required under 37 C.F.R. §§ 1.16 to 1.21 from the deposit account **23-2415**, referencing attorney docket number **26050-712.201**.

Respectfully submitted,

Date: August 16, 2005

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CHAPTER

3

Vectors for Phage Display

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A range of vectors are available for exogenous expression on the surface of bacteriophage M13 virus particles. The display sites most commonly used are within genes III or VIII, although there have been attempts at cloning in genes VII and IX (Makowski, 1993). Viral vectors that accept and display fusions for genes III and VIII have been termed type 3 and 8 (Smith, 1993), and are shown in Fig. 1. Many short peptides and a variety of proteins have been displayed at the N-terminus of mature pIII. pVIII, on the other hand, appears to tolerate only short inserts of five (Il'ichev *et al.*, 1989) or six amino acid additional residues (Greenwood *et al.*, 1991); this may be due to the close packed nature of the viral surface (Kishchenko *et al.*, 1994). Additional information regarding viral morphogenesis, pIII, and pVIII can be found in Chapter 1.

Many investigators have observed that some sequences are not well displayed on the surface of M13 phage, due to defects in viral particle assembly, stability, and infectivity. While most of this biological intolerance is unclear, two compensating vector systems have been designed. In one system, phagemid vectors carry a copy of either gene III or VIII; these vectors have been termed type 3+3 or 8+8 (Smith, 1993; Fig. 1). When bacterial cells harboring these phagemids are infected with M13 helper phage, which carry the full complement of capsid-encoding genes but are defective in replication, the secreted phage particles carry the phagemid genome and a mixture of wild-type and fusion pIIIs or pVIIIs (Fig. 2). In another system, the

Phage Display of Peptides and Proteins

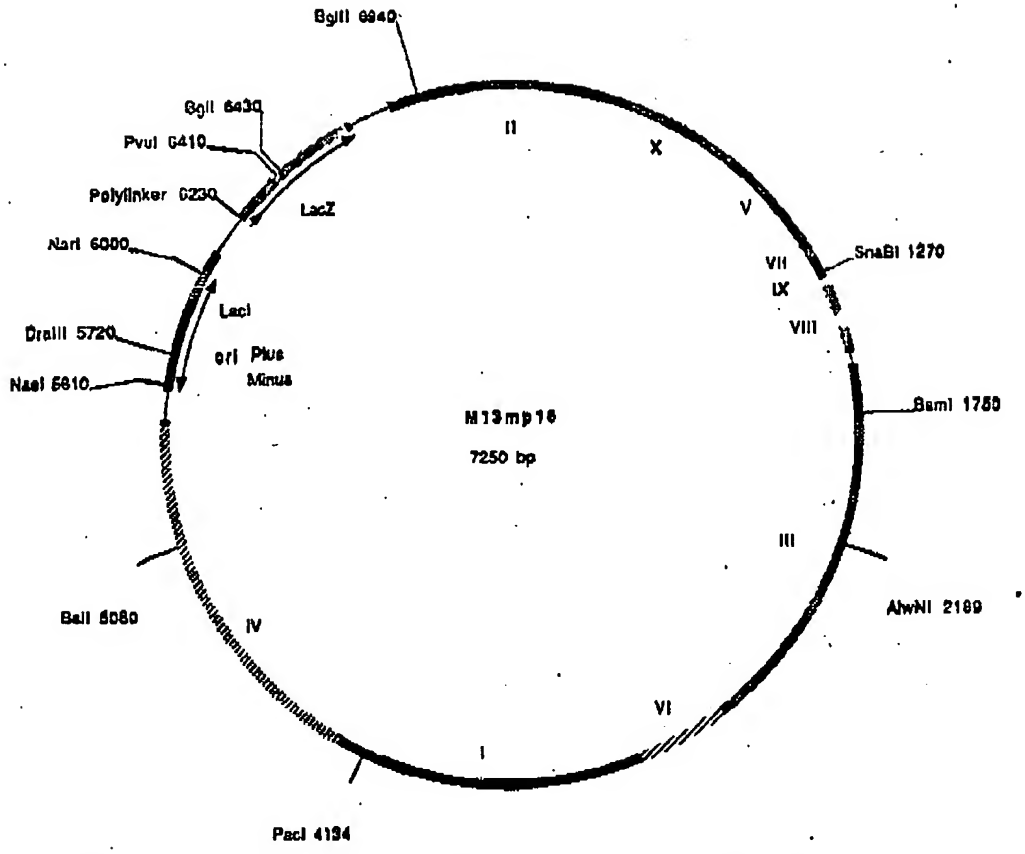

FIGURE 3 Map of M13mp18 genome. Unique restriction sites are shown on the circular map of M13mp18 (Accession No. X02513). The 10 viral genes are shown as boxes on the map; they are transcribed in a clockwise fashion. The polylinker within the *lacZ* gene contains the following restriction sites: *EcoRI*, *SacI*, *KpnI*, *SmaI*, *XmaI*, *BamHI*, *SalI*, *HincII*, *AccI*, *SphI*, and *HindIII*. The origin of replication can be in either orientation to direct the replication and packaging of either the plus or minus strand. The figure was generated with the program MacPlasmap, written by Jingdong Liu (Salt Lake City, UT).

ticles must also carry wild-type pIII, expressed from a second gene III in the vector (i.e., type 33) or a helper phage genome.

In several vectors, the displayed element is separated from the remainder of pIII or pVIII by short linkers. While there have been no formal experiments on the best linker sequences to use, many vectors use some variation of the sequence GGGGS. There are two glycine-rich regions in pIII (i.e., 68–86 and 218–256), consisting of many GGGS and EGGGS repeats. It is also possible to include a proteolytic cleavage site